

REMARKS

Claims 40-70 were pending in the above-identified application. Claims 1-39 have been cancelled previously, and claims 51-54 and 56-70 have been withdrawn from consideration by the Examiner as drawn to a non-elected invention. By this amendment, claims 51-54 and 56-70 have been cancelled as directed to non-elected subject matter. Applicants expressly reserve the right to prosecute the subject matter encompassed by any of claims 51-54 and 56-70 in a related co-pending application. Therefore, claims 40-50 and 55 remain pending.

In order to further expedite prosecution of the instant application and without acquiescence to any rejection of the Examiner or reason for rejection claims 40, 41, and 50 have been amended to more clearly set forth the present invention. Support for these amendments are identified in the following remarks. Further, claims 40 and 50 have been amended to correct clerical errors. In particular, the phrase "or vice versa" has been added back to claim 40 and the spelling of "side" has been corrected in claim 50. No new matter has been added by these amendments. Applicants request reconsideration of the claims currently pending in the application in light of the amendments above and the following remarks.

Rejections under 35 U.S.C. §112

Claims 40-50 and 55 remain rejected under 35 U.S.C. § 112, first paragraph, the Examiner believing that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with the claims. The Examiner contends that the specification, while being enabling for the use of retaining glycosidases where the catalytically active carboxylic acid that is a nucleophile is mutated to form an oligosaccharide using a specific glycosyl fluoride as a donor and a specific acceptor, does not reasonably provide enablement for a method of synthesizing any oligosaccharide using any glycosidase and any donor and acceptor molecules.

In setting forth the summary of the present invention the Examiner has interpreted the claims as being "so broad as to encompass [a] method of use of any glycosidase both retaining and inverting, in which any one of the two catalytic carboxylic amino acids is mutated, in a stereospecific reaction using any donor and acceptor." The Examiner does not believe the scope of the claims as interpreted by the Examiner is commensurate with the enablement provided by the disclosure with regard to the extremely large number of glycosidases having different structures, functions and substrate specificities, and donors and acceptors. Also, the Examiner believes the claims encompass any donor-acceptor pair irrespective of the original substrate specificity of a glycosidase. The Examiner asserts that "using any mutant glycosidase with any donor other than glycosyl fluoride and any acceptor is unpredictable and the experimentation left to the skilled artisan is unnecessarily and improperly extensive and undue. Therefore, it appears that the Examiner believes that the specification only enables one of ordinary skill in the art to use a retaining glycosidase mutated at a nucleophile catalytically active carboxylic acid with a respective glycosyl fluoride donor and a acceptor to form an oligosaccharide.

Applicants must respectfully traverse this rejection. First, as in the prior office actions in this application the Examiner has failed to make a proper rejection under 35 U.S.C. § 112, first paragraph. To make a rejection for enablement under 35 U.S.C. § 112, first paragraph, the Examiner has "the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention." MPEP § 2164.04, *citing In re Wright*, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Only once the Office has met this burden does the burden shift to the applicant to provide suitable proofs that the specification in enabling. Applicants do not believe that any reasoned statement, supported by scientific evidence, has been presented by the Examiner to establish a proper basis for an enablement rejection.

In the present office action, the Examiner has stated several factors pertinent to the inquiry for enablement. The factors recited included predictability of the art, guidance in the specification, breadth of claims and the amount of experimentation that would be necessary to use the invention. In making the rejection the Examiner has merely asserted that "the scope of

the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of glycosidases having different structures, functions and substrate specificities, and donors and acceptors broadly encompassed by the claims." This assertion provides no reasoned scientific basis for the present rejection.

In Applicants' prior responses, even though the Office had not met its burden in establishing a lack of enablement, evidence that the invention was fully described and enabled was provided in the form of a declaration and post filing published references. The declaration and references established that using the teaching and guidance provided by the present invention additional glycosidases differing in structure, function and substrate specificity had been mutated and, as expected, were found to be unable to hydrolyze oligosaccharide substrates of the wild-type enzyme, but were able to function as glycosynthases. The Examiner has reviewed this information and has analyzed the submissions narrowly as teaching "a few examples of other retaining glycosidases mutated at a catalytically active carboxylic acid nucleophile with a respective glycosyl fluoride as a donor." Further, the Examiner appears to now be concerned that only glycosyl fluoride donor species have been used. The Examiner concludes "that based on the disclosure and the state of the art it is unpredictable whether any glycosidase when mutated at any one of the two catalytically active carboxylic amino acids will catalyze coupling of any glycosyl donor and any glycoside acceptor having opposite stereochemical configurations by either [an] inverting or retaining mechanism." Again, no scientific reasoning is provided to support these assertions other than the number of glycosidases, acceptors and donors is large. The large number of possible elements that might be encompassed by a claim, by itself, is an insufficient basis for an enablement rejection.

Contrary to the narrow construction by the Examiner of the specification as filed and the evidence filed in support of enablement of the present invention, the specification as filed broadly teaches the mutation of glycosidases at one of two catalytically active amino acids in the active site of the enzyme results in an enzyme that has lost the ability to hydrolyse oligosaccharide products, but can catalyze the coupling of a modified glycosyl donor molecules to acceptors. The enzymes that have been specifically exemplified in the application as filed and

in the post filing references include, for example, a  $\beta$ -glycosidase, a  $\beta$ -glucosidase, a glucanase, a  $\beta$ -galactosidase, a  $\beta$ -mannosidase, an  $\alpha$ -amylase, an  $\alpha$ -glucosidase, and an endocellulase. These particular examples provide evidence that glycosidases having different structures, functions and substrate specificities can be mutated as taught by the present invention to obtain the expected result.

Further, the Examiner has not provided a reasoned basis for why the use of a specific donor species in the present application and in post filing examples does not establish enablement for the pending claims. The specification describes glycosyl fluorides as preferred species, but teaches that other groups which are reasonably small and which function as relatively good leaving groups can also be used. Examples of other glycosyl donor molecules provided included glycosyl chlorides, acetates, propionates, and pivaloates, and glycosyl molecules modified with substituted phenols. See page 12, lines 3 - 11 of the specification.

Although, Applicants do not believe that it is necessary to provide additional evidence of other donor species, but in order to further expedite prosecution, a declaration by one of the inventor, Stephen Withers, is provided which describes the use of alternative glycosyl donors other than glycosyl fluorides in transglycosylation reactions catalyzed by a mutant glycosidase of the present invention. In these experiments glycosyl formates and glycosyl azides were used as alternative donors and combined with a glycoside acceptor in the presence of a mutant  $\beta$ -glycosidase (*Agrobacterium* E358G). These glycosyl donors, although not specifically mentioned in the specification, would be recognized by the skilled artisan as being modified by a group that is reasonably small and which function as relatively good leaving groups as taught by the specification. The formate and azide modified glycosyl donors were synthesized *in situ* by the mutated enzyme itself, however, these donors can be independently synthesized by chemical means. The reactions were performed in a single vessel by combining an external nucleophile of the appropriate size (azide, formate, etc.) with an activated glycoside with the anomeric stereochemistry of the natural substrate along with the mutant glycosidase and a glycoside acceptor. The external nucleophile takes the place of the nucleophile removed from the active site of the mutated enzyme, with the added nucleophile replacing the activation group (DNP) of

the donor with inversion of anomeric configuration forming the modified glycosyl donor. In the second step of the reaction the activated glycoside donor was transglycosylated onto the acceptor to form the oligosaccharide.

In particular, dinitrophenyl- $\beta$ -D-glucopyranoside was incubated in the presence of either fluoride (KF), formate (NaHCO<sub>2</sub>) or azide (NaN<sub>3</sub>) with the nucleophile mutant *Agrobacterium*  $\beta$ -glucosidase (Abg E358G). Separately each reaction resulted in the formation  $\alpha$ -glucopyranosyl fluoride,  $\alpha$ -glucopyranosyl formate and  $\alpha$ -glucopyranosyl azide as indicated by an increase in the rate of dinitrophenol release as compared to that in a control reaction containing no external nucleophile. In the second (coupling) step of the reaction, each modified glycosyl donor produced in the first step was transglycosylated onto the acceptor *p*NP  $\beta$ -D-glucopyranoside. Thin layer chromatography experiments demonstrated that with all three modified glycosyl donors a transglycosylation reaction occurred as demonstrated by the formation of new UV-active compounds. Additional testing (ESI/MS) demonstrated that a *p*NP disaccharide and a *p*NP trisaccharide were formed when azide- and fluoride-modified glycosyl donor species were used as the donor species and a *p*NP disaccharide, a *p*NP trisaccharide and a *p*NP tetrasaccharide were formed when a formate-modified glycosyl donor species was used. Thus, it is evident that modified glycosyl donors other than glycosyl fluorides function in the present invention as set forth by Applicants in the specification as filed.

Further, in order to more clearly set forth the present invention, Applicants have amended claims 40 to recite a modified glycosyl donor. Support for this amendment is found, for example, at page 8, line 19 and page 12, lines 3-11. Also, claims 40, 41 and 50 have been amended to clarify that the carboxylic acid residues that are replaced are those of the catalytically active amino acids in the active site of the glycosidase enzyme. In claim 40 the amino acid replaced is "one of said catalytically active amino acids having a carboxylic acid side chain." While in claims 41 and 50, the phrase "one of the carboxylic acid side chains in the active site" has been replaced with the phrase "the carboxylic acid side chain of one of said catalytically active amino acids in the glycosidase enzyme active site." The phrase "other carboxylic acid side chain" has been replaced with "the carboxylic acid side chain of the other

catalytically active amino acid." None of these amendments narrows the claims in any way, but clarifies the nature of the carboxylic acid side chains and the amino acid residues function in the enzyme.

Applicants have taught that a glycosidase enzyme can be mutated at one of two catalytically active carboxylic acid side chain containing amino acids in the active site of the enzyme to produce an enzyme that no longer hydrolyzes an oligonucleotide substrate, but can transglycosylate a modified glycosyl donor and an acceptor glycoside. In the case of a retaining enzyme the normal nucleophilic amino acid within the active site is changed to a non-nucleophilic amino acid. While in the case of an inverting glycosidase, the mutant enzyme is one in which the amino acid which normally functions as a base is replaced by a non-ionizable amino acid. Further, Applicants have disclosed the catalytic amino acids that are replaced in a number of enzymes and have taught various methods for determining which amino acid is replaced for those enzymes that the catalytic amino acids may not have been identified. It should be noted, as discussed in the prior response, that the catalytic amino acids of a number of glycosidases were known at the date of filing the earliest claimed priority application, and that since that time the catalytic amino acids of a large number of additional enzymes have been determined by one or more of the methods disclosed in the application. Still further, Applicants have provided a number of post filing examples of mutated glycosidase enzymes that function as disclosed in the present application. With the present response Applicants provide examples of the use of modified glycosyl donor species in addition to glycosyl fluoride donors. Therefore, Applicants believe that contrary to the unsupported assertions of the Examiner, the present invention has been enabled for the full scope of the claims.

Applicants respectfully request the Examiner reconsider and withdraw the rejection of claims 40-50 and 55 under 35 U.S.C. § 112, first paragraph, in view of the amendments to the claims, the attached declaration, and the above remarks.

Double Patenting

Claims 40-50 and 55 remain rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 5,716,812. Claims 40-55 and 55 also remain rejected under judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 2 of U.S. Patent No. 6,284,494. As set forth in Applicants prior responses, the need for a terminal disclaimer will be evaluated and submitted if required one an indication that the claims are otherwise in condition for allowance has been received.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: 20 November 2003

By: Brian W. Poor  
Brian W. Poor  
Reg. No. 32,928

TOWNSEND and TOWNSEND and CREW, LLP  
Two Embarcadero Center, 8<sup>th</sup> Floor  
San Francisco, CA 94111  
Telephone: (206) 467-9600  
Telefax: (415) 576-0300  
35017232 v1